EFFECTS OF SEALANT CONDITIONERS ON OCCLUSAL SURFACE BACTERIA: A CLINICAL STUDY

by

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INTRODUCTION

This project was designed to clinically evaluate the effects of conditioning agents for pit and fissure sealants on the bacteria present in occlusal grooves and fissures in permanent molars in children.

One of the methods which is being currently advocated to prevent occlusal caries is the use of plastic sealants over the caries-susceptible pits and fissures of the occlusal surface of molars and bicuspids. It is known, however, that sealants can be placed inadvertently or on purpose over existing carious lesions on these occlusal surfaces. Little is known of what would then be the fate of existing microorganisms sealed into the pit and fissures and lesions on the tooth.

Part of the technique for placing the sealant is the preparation of the enamel surface with so-called conditioners which are weak acids. These conditioners are used primarily to etch the surface of the enamel in order to allow better retention of the sealant to the surface.

In this investigation one of two different conditioners or a control was applied to carious teeth to determine the effect of each conditioner on the bacteria present. When no viable bacteria was found after conditioning as tested by a culturing method, it was concluded that the conditioner had totally killed the bacteria. However, when viable bacteria remained following the conditioning procedure, it was concluded that the conditioner had not killed all the bacteria present. The purpose of the investigation was to gather information concerning the clinical usefulness of these conditioners.

REVIEW OF LITERATURE

Hennon, Stookey and Muhler[1] found that occlusal caries was the most prevalent form of dental decay in pre-school children. Ripa[2] also stated that more than 50 percent of all carious lesions in children were occlusal in nature. Many investigators have used various methods in the attempt to reduce the incidence of occlusal decay. Hyatt[3,4] in 1923 and again in 1928 suggested that a prophylactic odontomy be performed on occlusal areas of posterior teeth, with amalgam restorations being placed as a means of preventing dental decay. In 1929 Bodecker[5] suggested a similar technique. However, he only reduced the invaginated pits and fissures without placing an amalgam upon completion of this reduction. Neither Hyatt's technique nor Bodecker's was widely accepted by the dental profession.

From the 1940's to the 1950's, investigators used chemical reagents to reduce occlusal caries. Klein and Knutson[6] used ammonial silver nitrate in 1942, but no positive results in reducing decay were obtained. In 1947 Gottleib[7] swabbed silver nitrate on occlusal surfaces to prevent dental caries by closing the "invasive roads" of dental caries. Ast, Bushel and Chase,[8] following the work of Gottleib, used zinc chloride and potassium ferrocyanide, and again no positive effects were observed. In 1951 Miller[9] used red copper cement placed over pits and fissures and was also unable to show a reduction in occlusal caries.

In the 1960's researchers began to experiment with plastics, and occlusal pits and fissures were soon sealed with plastics in an attempt to reduce occlusal decay. One group of researchers headed by Buonocore and including Ripa and Cueto[10-12] conducted trials with plastic sealants and found them quite promising in regard to reduction of occlusal decay. After one year, more than 80 percent of the permanent molars and bicuspids sealed in 269 children of ages 5 to 17 years were caries-free while 42 percent of those not receiving the plastic sealant were carious. The difference between the groups was statistically significant, with $P > 0.05$.

With these initial positive results came many questions as to which would be the best kind of sealant and what would be the effect of widespread use of sealants. Answers to these questions began to unfold in the research of the 1970's, much of which is summarized here.

In 1972 the American Dental Association Council on Dental Materials and Devices classified one product, Nuva Seal by the L.D. Caulk Co., Milford, Delaware, as provisionally acceptable. This product is the only one so recognized and has been shown to have the most satisfactory bond strength to enamel.[13] This product is a bisphenol -A- glycidyl methacrylate sealant utilizing the catalyst benzoin methy ether which polymerizes upon exposure to ultraviolet light.

Even though this material is gaining in acceptance, many questions are still unanswered. In 1972 Katz, Stookey and McDonald[14] stated that "in most instances, sealants will very likely be placed over live bacterial colonies." They were unable to explain the clinical implications

of their statement but called for further study on the matter. They also stated that "perhaps the most important of these concerns refers to what will happen if a sealant is applied to a fissure already containing caries." In 1973 Swallow,[15] in summarizing work done on pit and fissure sealants, called for more clinical studies instead of "extropolating laboratory results." Phillips,[16] also in 1973, stated that much remains to be determined about pit and fissure sealants, and that previous studies were performed on teeth which "appear to be free of caries." In 1974 Lund[17] stated that in his opinion, a "conservative restoration is the best solution for grooves where caries is thought to exist". McDonald,[18] also in 1974, stated that "most of the studies reported thus far involve careful case selection and meticulous application of the material to surfaces which appear to be caries-free".

Cyanoacrylate Pit and Fissure Sealants

Three types of adhesive pit and fissure sealants are presently under investigation: the cyanoacrylates, (more commonly known as the "super-glues" used today); the polyurethane resins; and the bisphenol -A-glycidyl methacrylates.

In 1963 Buonocore [19] suggested a method of sealing pits and fissures of newly erupted teeth with a methyl-2-cyanoacrylate resin. He etched the occlusal surface with phosphoric acid and then applied the sealant. He stated that this would allow for long-lasting, water-resistant bonding to the tooth.

Later Buonocore, with Cueto,[20,21] reported on a one-year study in which a filled, methyl-2-cyanoacrylate sealant was applied to the

occlusal enamel of newly erupted permanent molars and bicuspids. The teeth were etched for 60 seconds with 50 percent phosphoric acid containing 7 percent dissolved zinc oxide to simulate private practice recall procedures, the cyanoacrylate was applied a second time six months later. In a comparison of the caries on the experimental and control teeth, an 86 percent reduction was noted on the sealed teeth. Reporting after two years, Ripa with Cueto and Buonocore[22] still found 86 percent less occlusal caries on the sealed teeth.

These first clinical trials produced encouraging results and they were followed by others by Takenchi and his associates[23-25] who reported up to 100 percent caries reduction after two years and 91 percent reduction after four years. These high percentages were attributed to the careful selection of teeth with no occlusal decay.

In 1969 and 1970 Ripa and Cole[26,27] reported over 80 percent caries reduction in a twelve-month study using cyanoacrylates. However, in 1971 Parkhouse and Winter[28] had almost 100 percent failure in their six-month clinical study using a form of cyanoacrylate.

In 1972 Pugnier[29] reported more favorable results with some 50 percent reduction in decay using cyanoacrylates in a two-year study.

In 1973 Buonocore[30] summarized the intrinsic problems in the use of cyanoacrylates. He provided information on their difficulty in handling and their poor resistance to decomposure by hydrolysis in the oral cavity.

In 1974 the Council on Dental Materials and Devices of the American Dental Association[31,32] issued a summary opinion on the current status of cyanoacrylate resins. Based on its findings, the cyanoacrylate-based

resins are not acceptable as pit and fissure sealants in clinical dentistry at present.

Polyurethane Pit and Fissure Sealants

Another adhesive system under study is the polyurethane resin sealants, and Epoxylite 9070[a] is the most widely-tested sealant in this category. Lee and Swartz[33] in 1971 noted that this system trapped air in the deepest fissures, and they suggested modification of the Epoxylite.

In 1972 Rock[34] was unable to show significant reduction in occlusal decay in a one-year study using Epoxylite 9070, and reported nearly total loss of this sealant from the occlusal surfaces after one year. Rock concluded that due to their extremely low ability to seal, the polyurethane sealants were unacceptable when used alone for clinical sealing of pits and fissures to prevent caries.

Bisphenol-A-Glycidyl Methacrylate Pit and Fissure Sealant

The most promising system to date is the bisphenol-A- glycidyl methacrylate pit and fissure sealant. Better known as BPA-GMA, this system was described by Bowen[35,36] and has since been studied extensively by many investigators.

A pilot study by Roydhouse[37] in 1968 of 130 children with caries-free first permanent molars showed almost a one-third reduction in decay over a three-year span.

In 1970 Buonocore[38] reported on a modification he had made in the BPA-GMA catalyst system, and his results over a one-year period

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a. Lee Pharmaceutical Company, South El Monte, California

showed no loss of sealant and no decay in the 200 tested teeth, but 42 percent decay in the occlusals of the control teeth. Buonocore had substituted benzoin methyl ether for the previously used benzoyl peroxide, an alteration which resulted in a material that would set under exposure to ultraviolet light. He also used 50 percent phosphoric acid to etch the enamel to increase retention of the BPA-GMA system.

Buonocore[39] and later Buonocore and Gwinnett[40-42] further demonstrated that using phosphoric acid does indeed etch the enamel and increase retention.

In 1973 Chow and Brown[43] further investigated the amount of phosphoric acid to be used and found that the 50 percent level was conducive to best adhesion. They found that a higher percent of phosphoric acid caused less etching due to a protective film formation of $Ca(H_2PO_4)_2$-H_2O. A lower percent of phosphoric acid left a salt which would not be completely washed away.

The commercial product Nuva Seal[a] is a BPA-GMA system polymerized with ultraviolet light and is presently the most thoroughly studied sealant system. Buonocore[44] reported excellent two-year clinical results with this material. Using a single application, he was able to show retention of the sealant in over 80 percent of the 113 permanent teeth and 50 percent of the 40 primary teeth.

In 1971 McCune and Cvar[45] and again in 1973, McCune and associates[46] reported that 90 percent of the BIS-GMA sealants were retained during a one-year period and that there was a reduction of nearly 90 percent in the dental caries rate.

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a. L.D.Caulk Co., Milford, Delaware

In 1973 Buonocore[47] summarized his work to date by referring to the concept of pit and fissure sealants as "...an important adjunct in caries prevention programs since it is intended for those caries-susceptible areas least benefitted by fluoride".

Robb and Garcia[48] reported on clinical observations of a chemically cured BIS-GMA sealant, Epoxylite 9075,[a] and a fluoride-containing polyurethane resin, Epoxylite 9070,[a] both in fluoridated and non-fluoridated areas. They observed that after twelve months, there was complete retention of 97 percent of the sealants on permanent teeth treated with BIS-GMA resin. As in earlier studies, retention was poor in primary teeth due to their anatomical lack of well defined pits and fissures.

Newhouse and Roydhouse[49] investigated the penetration of chemically cured BIS-GMA sealant into the pits and fissures both in vivo and in vitro. Their findings microscopically were nearly complete penetration to the base of the pit or fissure of the sealant.

In evaluating the clinical performance of two BIS-GMA sealants, Rock[50] used a similar technique of etching occlusal pits and fissures in permanent teeth and sealing them with one of the sealants. The sealant which was chemically activated displayed retention of only 59 percent after six months and only 53 percent after twelve months. The other sealant, activated by ultra-violet light, was retained in 91 percent of the teeth after six months and in 86 percent after twelve months. Even though the retention varied, both sealants showed significant reduction in incidence of occlusal caries.

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a. Lee Pharmaceutical Company, South El Monte, California

Based upon extensive study of bisphenol-A and glycidyl methacrylate resin for sealant use, the Council on Dental Materials and Devices issued reports[51,52] in 1972 and 1973 which allowed provisional acceptance of Epoxylite 9075 and Nuva Seal.

In 1975, Burt and coworkers[53] found that of 427 permanent teeth sealed with Nuva Seal, and with contralateral teeth as controls only 39 percent had full sealant retention after six months. Due to a wide age range of five to seventeen years, and the fact that 75.2 percent of the sample consisted of molars (which have less retentive success than bicuspids), the researchers felt that they had less success than had been previously reported. Also the use of younger, less cooperative children didn't allow for the best possible technique.

Also in 1975 Cons, Pollard, and Leske[54] tested Nuva Seal for caries inhibition and for retention when used as a pit and fissure sealant on first permanent molars of children in a water-fluoridated community. After twelve months, only 77 percent of the teeth had sealant on them. At the end of 24 months, the retention of the sealant had dropped to 50 percent.

Acid-Etching of Enamel

As early as 1955, Buonocore[55] had demonstrated that for increased marginal seal and increased retention of acrylic resins to enamel, a treatment of the enamel surface with a dilute acid would be advantageous. The tooth surface was treated with 85 percent phosphoric acid for 30 seconds, which was thought to be a clinically safe procedure since the concentration of phosphoric acid was similar to that already in use in

zinc phosphate dental cement. White or opaque changes were observed on the treated enamel, but these disappeared after a few days.

Later, Gwinnett and Matusi[56] examined the resin enamel interface microscopically. The enamel which had been treated with acid had tag-like extensions of resin which aided retention mechanical and microscopic interlocking with the enamel prisms. This mechanical bond with enamel decreased the susceptibility of the surface to demineralization even when the bulk of the resin was lost.

Buonocore, with Matusi and Gwinnett,[57] used 50 percent phosphoric acid with 7 percent dissolved zinc oxide to treat the enamel of a number of specimens for sixty seconds each. They then placed various resins on the different treated surfaces and noticed increased mechanical bonding of the resins to the enamel for periods of up to one year. The bonding resulted in prism-like tags of resin up to 25 microns in length.

Sheykholeslam, with Buonocore[58,59] reported in 1970 and again in 1972 that when 50 percent phosphoric acid with 7 percent zinc oxide was used on permanent and primary teeth, there was a difference in retention due to an increase in mechanical tags. The facial surfaces of primary teeth, where there is a prismless enamel, showed an almost total lack of tags of resin in the enamel.

Laswell, Wells and Regenos[60] reported on the clinical use of acid-etching procedures relative to operative dentistry. Treating the enamel surface with 50 percent phosphoric acid for 60 to 120 seconds improved the retention of unfilled acrylic resin restorations to the enamel.

Lee, Phillips, and Swartz[61] demonstrated on bovine enamel in vitro that using 50 percent phosphoric acid for sixty seconds to condition the surface increased the bonding of unfilled resin as measured by a tensile test. This bond was significantly stronger when a cavity sealer was applied after etching of the enamel.

In 1972 Gwinnett and Buonocore,[62] with the use of the scanning electron microscope, studied the enamel surfaces of extracted permanent teeth which had been conditioned for sixty seconds with 50 percent phosphoric acid containing 7 percent dissolved zinc oxide. They reported that the increase in enamel porosity was confined almost exclusively to the cuspal incline planes. The conditioner was ineffective in etching below the fissural constrictions. The authors said that this was probably the result of debris at the bottom of the pits and fissures. They noted further that the debris resembled microorganisms as they were spheroidal and rod-shaped bodies.

Silverstone and Snewin[63] evaluated a number of solutions in vitro as to the ability to etch human enamel. One of the solutions, phosphoric acid, was tested in concentrations from 20 percent to 70 percent, with time of application varying from thirty seconds to ten minutes. For phosphoric acid, the degree of surface etching decreased as the concentration increased.

In 1973 Gwinnett[64] compared the effect of etching the "prismless" enamel which is characteristic of primary teeth to the effect of etching the prismatic enamel. Using resin, he noted that there was little porosity and penetrability in the "prismless" enamel and thus only slight

resin penetration compared to the etched prismatic enamel. He explained that this lack of mechanical bonding probably accounted for the lower retention incidence of resins to primary enamel.

In 1973 Retief[65] reported on 50 percent phosphoric acid etching of human enamel in vitro. He recorded resin tags projecting 50 microns into the conditioned enamel. The conditioning was said to increase the surface wettability of the enamel, allowing for attack at the mineral phase of the hydroxyapatite crystal. Retief further noted almost complete recovery of the surface within fourteen days.

In 1974 and 1975 Silverstone[66,67] reported that for various sealants, the greatest amount of enamel etching was produced by 30 percent phosphoric acid. In his in vitro retention study, he noted that not all sealants maintained a resin-enamel interface to the same degree when viewed by the scanning electron microscope.

Also in 1975, McLundie and Messer[68] found in their laboratory study that 30 percent unbuffered phosphoric acid seemed to cause slightly more roughening of the enamel surface than did the buffered acids now used with some resin systems. They were, however, able to see the etched prisms clearly for all specimens.

Rakow, Chertoff, and Light[69] pointed out in their article on the pitfalls of acid-etch techniques that dilution of the conditioner by moisture can lead to inadequate demineralization of the enamel during etching.

Jorgenson and Shimokobe[70] used 35 percent orthophosphoric acid to etch the enamel of extracted human teeth. They noted that this concen-

tration allowed for good adaptation of resins, both filled and unfilled, to the etched enamel surface.

In 1975 Retief[71] compared the use of 50 percent phosphoric acid with the use of 50 percent phosphoric acid attenuated with 7 percent zinc oxide, and with the use of citric acid in 50 percent concentration. Both the attenuated and unattenuated phosphoric acid in 50 percent concentrations elicited comparable etching action when viewed with the scanning electron microscope. The 50 percent citric acid solution had a much milder etching action.

It is apparent that when etching with dilute acids the surface should be kept as dry as possible to enable the reactive layer of the etched enamel to form a complete interface with the resin product. It is also noteworthy that in concentrations of 30 percent to 50 percent phosphoric acid has been shown to successfully etch prismatic enamel, at least down the incline planes of the pits and fissures.

Sealing Properties of Pit and Fissure Sealants

It now appears that research has afforded us materials which can penetrate the pits and fissures and seal the underlying enamel and dentin from the oral environment.

Fan, Seluk, and O'Brien[72] in 1975 reported that the ability of sealants to penetrate into grooves and fissures is a function of the properties of the sealant, the surface tension, the viscosity, and the contact angle of the capillary wall. They also noted that the whole process is temperature dependent.

In 1972 Rudolph,[73] using an in vitro assessment of the microleakage of pit and fissure sealants, found that they do indeed seal the tooth from the oral environment. He observed that pit and fissure sealant materials should not fail clinically because of leakage.

Another thesis, by Eliasson,[74] in 1974 reported on the sealing properties of fluoride-containing pit and fissure sealants. He concluded that, with the exception of the cyanoacrylates, adding sodium fluoride to the sealants did not alter their excellent sealing qualities.

In an in vivo follow up of Eliasson's work, Wilkins[75] found that the retentive qualities of the BIS-GMA resin were unaffected by addition of the sodium fluoride. Since the monkey was used as a model, the clinical effects of sealing with the fluoride versus the non-fluoride sealant were inconclusive.

We are now relatively certain that a well placed BIS-GMA sealant will effectively seal a pit and fissure clinically, but we are uncertain what adding reagents might do to this sealing ability.

Sealed Bacteria

The ability to seal a pit and fissure from the outside environment has been established, but what happens under the sealant is yet to be determined. The primary concern here is what becomes of the sealed bacteria under a sealant.

Besic[76] found that under dental restorations, sealed bacteria remained viable up to a year.

Schoube and McDonald[77] also found that organisms remained viable for long periods under some dental restorations.

King, Crawford, and Lindahl[78] demonstrated that the number of cultivable organisms was reduced when deep carious dentin was sealed under indirect pulp-capping materials.

Handleman and associates[79,80] demonstrated in a number of published studies from 1969 to 1972 that the number of microorganisms in carious dentin sealed with the BIS-GMA system was substantially lower than on the control contralateral teeth. However, some microorganisms remained viable, which led Handleman and Buonocore[81] in 1973 to recomment that "...clinical studies with additional bacteriologic evaluation, as well as studies to determine possible changes in the dentin should be done."

Newbrun, Plasschaert, and Konig[82] demonstrated in an experiment that mean caries scores were higher for carious rats' teeth treated with a pit and fissure sealant than for those which were not so treated. This finding led them to state that "...the fate of initial carious lesions inadvertently sealed beneath these plastics merits further in vivo investigation."

Mednick, Loesche, and Corpron[83] in 1974, sealed bacteria in the primary teeth of children, and concluded that "...some bacteria did remain viable, and presumably retained a potential for pathogenicity."

El-Kafrawy and Mitchell,[84] studying the effect of sealants on caries progression in rats, were unable to note any effect in delaying caries progression. Using 186 Wistar rats' teeth with untreated teeth as controls, and using composite and cyanoacrylate resins, they were unable to obtain adherence to pre-existing caries. Thus, caries progress apparantly was unaffected.

Jeronimus, Till, and Sveen[85] in 1975 reported on sealing bacteria in occlusal lesions on children's permanent molars with certain pit and fissure sealants for periods up to four weeks. The cultivable bacteria from dentin samples appeared to decrease on some of the samples. The authors also attributed some immediate lack of bacteria in certain cultures to phosphoric acid contamination resulting in a sterile lesion to start with. Interestingly, the incipient lesion which extended to the deepest limit of the authors' "incipient" classification (radiographically) did not show reduction of the viable bacteria in the carious dentin after sealing.

The fate of entrapped bacteria under sealants on carious teeth is an unanswered question. Information is needed on what effect, if any, the acid conditioning plays in reducing the viability of the microorganisms for which we are testing. This study investigated those effects.

METHODS AND MATERIALS

This investigation was designed as a clinical study of children approximately eight through twelve years, each of whom was a patient of the Indiana University School of Dentistry, Indianapolis, Indiana. At the onset, the legal guardian of each child was required to sign a consent form authorizing the child's participation (Appendix). Each of over 100 patients was diagnosed by one of the examiners as having at least one permanent molar (regardless of location in the mouth) with occlusal dental caries with no previous restorations on the occlusal surface, and less than one millimeter of lateral extension of caries outside that of normal pits and fissures on the tooth. No lesion was accepted which had radiographic evidence of carious involvement of more than 50 percent of the dentin from the dentino-enamel junction to any point in the peripheral outline of the pulp chamber. The total sample size contained approximately 100 such teeth. Posterior bite-wing radiographs were taken at no charge to the patient, and any tooth shown to have interproximal caries was excluded. Occlusal caries was interpreted, as described by McDonald,[86] as those pits and fissures in which the sharpest exploring point will stick. This interpretation is also part of the definition put forth by the American Dental Association.[87] The teeth selected for study were randomly distributed into the following three groups.

1) Group 1 - those teeth in which the occlusal surface would be conditioned with distilled water for sixty seconds.

2) Group 2 - those teeth which would be conditioned with 50 percent aqueous phosphoric acid for sixty seconds.

3) Group 3 - those teeth which would be conditioned with the conditioner from the L. D. Caulk Company containing 50 percent phosphoric acid and 7 percent zinc oxide.

The teeth were referred to by patient number and the Universal Numbering System for Human Adult Dentition.[88] The operator was unaware of which group he was dealing with or which conditioner he was applying. Each conditioner was placed in an amber glass bottle and labelled by means of a coded system.

A sterile rubber dam was used for patient comfort and protection. There was pre-sterilization of the rubber dam sheet which had been pre-punched, laced with a #14 Ivory clamp, attached to the rubber dam frame, and the clamp holder locked into the clamp. The teeth were covered with the patient's saliva and dried to insure normal conditions before conditioners were placed. All instruments, burs, and handpiece were sterilized, and all procedures, including application and removal of the conditioners, followed aseptic techniques. The patients were randomized to prevent bias. To prevent identifying the agent, the bottles used were brown amber filled to exactly the same level. Bias was further prevented by randomly numbering reagents and coding them for the purpose of making this a blind study (Appendix). A standard tray set up (Figure 1) was arranged and sealed in a plastic bag by searing the ends of the bag with heat. It was then sterilized by steam autoclave, and the instruments were used for only one tooth. All rubber gloves were of the disposable, pre-sterilized, surgical rubber type

and were used by the operator and assistant only for a single tooth culturing operation.

All five hundred tubes were sterilized after culture media had been placed in them. Only small groups of tubes were handled at any one time so that no accidents would occur during the transfer process. All tubes were double-labelled with waterproof black ink so that neither the random number nor letter would be misread or wiped off during handling. All tube ends were flamed in an alcohol flame upon opening and closing. Tube caps were held in the gloved hand so as to prevent their contamination. The duration that the cap was off each tube was considered critical, and was held to a few seconds. The operator wore sterile rubber gloves at all times. Each type of conditioner was placed over carious lesions as specified in the directions designated by the L. D. Caulk Co., Milford, Del. for its commercial conditioner Nuva Seal. After the teeth were isolated, their carious grooves and fissures were wiped with a #3 cotton pledget moistened with sterile water. This pledget was then placed under sterile conditions into a test tube containing brain-heart-infusion broth with 0.02 percent thioglycollic acid. The tube was labelled with the tooth's assigned random number and the letter A representing the initial culture from the tooth.

The conditioner assigned to the tooth unknown to the operator, was then applied to the grooves and fissures for sixty seconds with a sterile #3 cotton pledget. The conditioner was then washed away with ten drops of sterile distilled water from a dropper with the excess being drawn up by high-speed evacuation. The aspiration instrument

was not allowed to touch the tooth surface. The tooth then had its occlusal grooves and fissures wiped with a #3 sterile pledget moistened with sterile water and the pledget was then placed in a test tube with brain-heart-infusion broth containing 0.02 percent thioglycollic acid. This second tube was labelled with the assigned random number of the operated tooth and also contained the letter B for the post-treatment designation.

Next the cavity preparation for a Class I carious lesion was begun, with the removal technique standardized to improve efficiency, reduce the degree of error, and minimize the number of variables. The carious enamel was removed with a sterile #331L burr with high-speed suction employed to eliminate debris. The dental caries in all cases was removed slowly by the same operator until the clinical depth of the caries was visualized. A third cotton pledget moistened with sterile distilled water was placed to the depth of the preparation and held for about four seconds. The pledget was then withdrawn and placed in a test tube with the brain-heart-infusion broth containing 0.02 percent thioglycollic acid. This tube was then labelled with the random number assigned to the operated tooth and the letter C representing the culture at the cavity depth after treatment.

The cavity preparation then proceeded. All preparations were completed for Class I amalgam restorations which were placed at no charge to the patient, and the patient was released from the study. The three tubes for each tooth were then incubated in a closed container with a Gas Pak (hydrogen and carbon dioxide generator) for three days at 37

degrees Centigrade. After this time, the cultures were observed and the results were recorded as positive or negative growth as judged by the culture's turbidity (Appendix, Tables I - XIV). The cultures were incubated aerobically another 24 hours at 37 degrees Centigrade, with these results also being recorded. All negative results were double-checked by subculturing after the fourth day. A statistical analysis was computed utilizing the chi square[89] test.

RESULTS

A total of 119 appointments were made for patients who met the requirements of this study. Ninety-eight of these were kept, fourteen were canceled in advance, and seven were failed by the patients.

Randomly assigned Group 1 which had the largest usable sample size (34 teeth) was distilled water. This group had 100 percent positive cultures at all levels of culturing. These 34 teeth which were conditioned with distilled water comprised 39.5 percent of the total sample size of 86. The 23 teeth conditioned with 50 percent aqueous phosphoric acid was the smallest of the groups and had the fewest positive cultures at all levels, with 60.9 percent (14 of the 23) having continued growth at all three levels. Twenty-five percent had no growth of the surface bacteria (Test A) after conditioning and 21 percent had no growth of bacteria at the depth of the lesion after conditioning with 50 percent phosphoric acid. The final set of samples were conditioned with 50 percent phosphoric acid with 7 percent zinc oxide. It totalled 29 usable teeth. Of the 29 teeth, 19, (65.5 percent) displayed culture turbidity which indicated positive growth at all three testing levels. Five of the 29, (17 percent) had no bacterial growth on the surface after conditioning. At the depth of the lesion (Test B), there were six cultures which were negative: that is, 20 percent of the 29 samples, which showed no growth after conditioning with this conditioner. Of the 29 samples in which the conditioner composed of 50

percent phosphoric acid with 7 percent zinc oxide was used, 11 showed some inhibition of bacterial growth on at least one level of testing. This was only 34 percent of those treated with this conditioner.

The results as summarized in Table I demonstrate that in the three groups, there were 86 total usable samples, 34 in Group 1, 23 in Group 2, and 29 in Group 3. Table II shows the incidence of positive growth at each testing level (A, B) and for each of the three conditioners.

In summary, the results of the chi square test demonstrated no significant difference in the distribution of positive bacterial cultures from occlusal surfaces treated with these three conditioning agents.

(x^2=.31, df=2, a=.05)

TABLES AND FIGURES

TABLE I

Effects of three conditioning agents upon the presence of viable bacteria on the occlusal surface and depth of occlusal caries (all the specimens gave positive cultures on the occlusal surface prior to the application of the conditioners)

Group	Conditioner	Number of teeth* and culture results				
		Occlusal surface		Depth of lesion		Total number of teeth
		Positive	Negative	Positive	Negative	
1	Distilled Water	34	-	34	-	34
2	50% phosphoric acid	17	6	18	5	23
3	50% phosphoric + 7% zinc oxide (Nuva seal)	24	5	23	6	29
Grand Total		75	11	75	11	86

* Excludes non-usable data

TABLE II

Number of positive cultures

Conditioner	A	B	Total
Distilled Water	34	34	68
50% aqueous phosphoric acid	17	18	35
50% phosphoric acid with 7% dissolved zinc oxide	24	23	47
Grand Total	75	75	150

A - post-conditioned occlusal surface

B - post-conditioned depth of lesion

TABLE III

Statistical analysis for positive cultures (chi square test)

Conditioner	A Observed	A Expected	B Observed	B Expected	Total Cultures
Distilled water	34	32.5	34	32.5	68
50% aqueous phosphoric acid	17	18.5	18	18.5	35
50% phosphoric acid with 7% dissolved zinc oxide	24	24	23	24	47
Total	75	75	75	75	150

A - post-conditioned occlusal surface

B - post-conditioned depth of lesion

A chi square test shows that there were no significant differences between the conditioners on either the occlusal surface or depth of lesion observations.

$x^2 = .31$ df = 2 $\alpha = 0.05$

TABLE IV

Comparison of Positive and Negative Cultures from the Occlusal Surface

Conditioner	Cultures				Total
	Positive		Negative		
	Observed	Expected	Observed	Expected	
Distilled water	34	30	0	4	34
50% phosphoric acid	17	20	6	3	23
50% phosphoric acid + 7% zinc oxide	24	25	5	4	29
Total	75	75	11	11	86

A chi square test shows that there were significantly more positive than negative cultures.

$$x^2 = 8.27 \qquad df = 2 \qquad \alpha = 0.05$$

TABLE V

Comparison of positive and negative cultures from the depth of the lesion

Conditioner	Positive Observed	Positive Expected	Negative Observed	Negative Expected	Total
Distilled water	34	30	0	4	34
50% phosphoric acid	18	20	5	3	23
50% phosphoric acid + 7% zinc oxide	23	25	6	4	29
Total	75	75	11	11	86

A chi square test shows that there were significantly more positive than negative cultures.

$$x^2 = 7.22 \qquad df = 2 \qquad \alpha = 0.05$$

FIGURE 1. Standardized tray set up for culturing.

- Heat-sealed autoclave bag
- Sterile gauze
- Cotton applicators
- Cotton pliers
- Sterilized cotton pledgets
- Pre-punched rubber dam with clamp and forceps attached

FIGURE 2. Photograph demonstrating positive growth culture with turbidity and cotton pledget after incubation.

FIGURE 3. Photograph illustrating negative growth in culture following incubation period with cotton pledget.

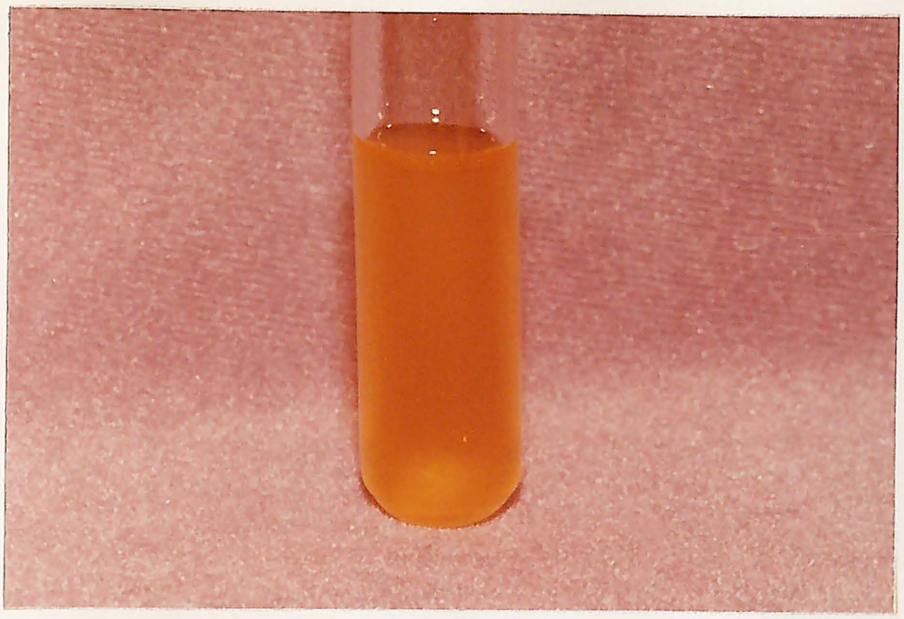
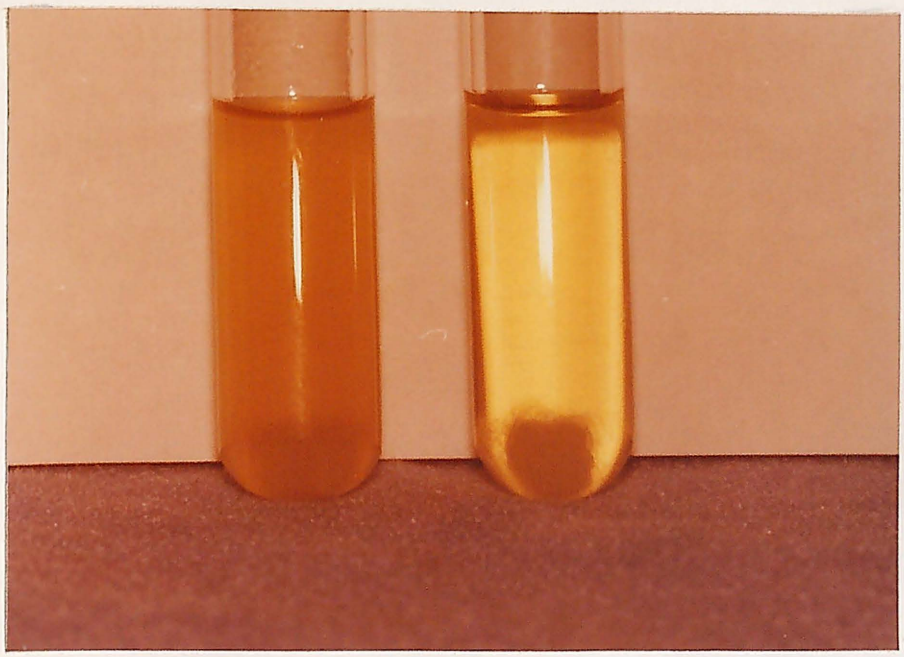


FIGURE 4. Photograph demonstrating positive culture on right and negative culture on left after incubation and read with white backround.



DISCUSSION

This study was designed to determine the different effects which sealant conditioners would have on the bacteria present in occlusal surface lesions. The variables were limited to increase the reliability of the results. The technique was standardized to allow for as little culture contamination as possible.

In any bacterial growth study with a sensitive medium, contamination from the environment is the hardest factor to reduce. Both the operator and the assistant used as little motion as possible in the area of an open culture tube. One complicating factor which future techniques can refine was the tendency of the serrations on the cotton pliers to prevent the cotton pledget from falling freely into the test tube. This delayed holding, occasionally made it necessary to shake the pliers, which increased the exposure of the cotton to the environment. Even though this problem was noticed early in the study, no attempt was made to change the technique since it was our objective to keep the entire study standardized. A simple solution for future techniques would be to use cotton swabs and break the tip of the stick off in the test tube.

Since the sequential technical procedures for this study had not been previously tested, this investigation was considered a pilot for future research in culturing carious lesions. The results were consistent from day to day as can be seen in the Tables in the Appendix. A further sign of confidence in the technique is the fact that the cultures of the pre-conditioned occlusal surfaces were one hundred percent

positive. This, of course, would be expected from the salivary bacteria present in addition to the bacteria present in the occlusal opening of the lesions. Also supporting the reliability of the culture technique is the fact that one hundred percent of cultures from teeth treated with the placebo control, distilled water, were positive.

As shown in Figures 2 and 3, positive and negative growth after four days of incubation was easily identified visually. To further aid in the identification, a white backround was used as in Figure 4 to allow for a clear delineation.

The culture readings were aided by the fact that after three days of culturing, the cotton pledget would float to the top of the test tube media when positive growth had occurred. This could be explained by the gases given off during bacterial growth pushing the cotton pledget up. The floating cotton would, of course, sink if the gases were released by shaking.

Eight conditioned teeth had positive bacterial cultures on the surface and negative cultures at the depth of the lesion. The reason for this is unknown.

There were eight additional teeth which had negative surface bacterial cultures but had positive depth of lesion cultures. These subsurface bacteria possibly were never reached by the reagent due to poor wettability of the tooth by the agent or due to physical restraint of the fissure and debris.

Only three of the 86 teeth had their bacteria killed at both the surface and the depth of the lesion. The statistical analysis allowed

us to conclude that the placebo and two conditioners were not significantly different in their ability to prevent bacterial viability in occlusal lesions. Approximately one third of the teeth in each of the acid reagent groups demonstrated at least one negative culture. However, as a clinical tool to prevent further bacterial viability, conditioning acid would be of little value since it is effective in less than forty percent of the cases. The slight difference in the two reagents was not significant.

When future studies are conducted in the field of pit and fissure sealants in the presence of suspected occlusal decay, the near lack of effect of the phosphoric acid conditioner should be taken into consideration. If an increased effect on the viability of the bacteria is to be obtained, better access to the bacteria by the acids must be devised or antibacterial agents must be introduced which have a more profound and reliable effect.

The testing method was such that only total kill or the lack of total kill of the bacteria was tested. The conditioner being tested could have actually killed a vast majority of the bacteria present, but the remaining viable bacterial cells could have caused the observed positive growth. It was thus concluded that there were bacterial cells which remained present but no measure of the amount was taken.

In previous observations in the laboratory, the author noted that small amounts of phosphoric acid added to a test tube of growing microorganisms were able to totally kill the bacteria probably due to a decrease in the pH. In this study, however, the conditioners were prob-

ably unable to make contact with all the bacteria present. This could have been due to the physical restriction of the narrow pits and fissures or the limited amount of time during which the conditioner was applied as related to its depth of penetration into the mass of bacteria.

When considering sealing over occlusal caries with pit and fissure sealants, it would be safe to say that the conditioning agent used does not kill all the bacterial cells present and thus bacteria are being sealed over and trapped in the lesion.

Better access to the bacteria by the acids could be obtained by forcing the conditioner into the carious pits with a sharp instrument or by increasing the conditioning time or decreasing surface tension. Another possible alternative would be to increase the conditioner's effectiveness by adding an antibacterial agent which might have a more reliable and profound effect.

SUMMARY AND CONCLUSIONS

A clinical study was conducted on the effects and clinical usefulness of conditioning agents for pit and fissure sealants on the bacteria present in carious occlusal grooves in permanent molars. Three conditioning agents, 50 percent phosphoric acid, 50 percent phosphoric acid attenuated with 7 percent by weight of zinc oxide, and distilled sterile water were compared for their ability to kill bacteria in carious lesions.

Ninety-five teeth were sampled for the study with a culture method that uses a medium of brain-heart-infusion broth with 0.02 percent thioglycollic acid. Results were measured as to growth or no growth after incubation at periods of three and four days.

Several problems associated with this method undoubtedly influenced the results. These included the inability to control the accessibility of the reagents to the pits and fissures of the tooth and the inability to control environmental contamination. In spite of these problems, the results were consistent, and the data justify several conclusions.

The culture method used was reliable and could be used when the presence of bacteria in clinical studies needs to be tested.

There was no statistically significant difference in the ability of the two acids and the water placebo to totally kill bacteria on the occlusal surface of the teeth studied.

The two pit and fissure conditioners did not totally kill the bacteria in occlusal lesions often enough to justify their use as bactericidal agents.

APPENDIX

INDIANA UNIVERSITY SCHOOL OF DENTISTRY
GRADUATE PEDODONTICS
1121 West Michigan Street
Indianapolis, Indiana 46202

Phone: 264-7952

Dear Parent:

Your child has been selected to participate in a program designed to provide information which will help dentists choose the best material to prevent tooth decay in commonly involved grooves of young permanent teeth. We would like to obtain your cooperation and that of your child in this program.

An examination indicates that your child has at least one permanent molar with a cavity on the biting surface. We will X-ray this tooth at no charge to you. We will then place a thin liquid coat in the grooves of the biting surface of the tooth. This short term liquid coating will not be harmful to the tooth, and your child should feel no discomfort from this procedure. After sixty seconds, we will remove the liquid coat and place the needed silver filling by the usual manner in your child's tooth at no charge to you.

Your child will benefit from participation in this program by having a cavity filled at no cost to you. Of course, we can provide this service only to those teeth selected for study in this program. Dental care for the other teeth should be arranged as in the past. Authorization by you for your child's participation is of course voluntary, and you may withdraw your child at any time. Feel free to ask any questions about our program.

Please sign your name below if you wish your child to participate in this program. Thank you in advance for your assistance.

_____ _____

Parent (legal guardian) Relationship

_____ _____

Your child's name Date

_____

Witness

Coded Key to Unknowns

A CLINICAL STUDY OF THE EFFECTS OF
CONDITIONING AGENTS FOR PIT AND FISSURE
SEALANTS ON THE BACTERIA IN OCCLUSAL CARIES

Group I Distilled Sterile Water ----- #__3__

Group II 50% Aqueous Phosphoric Acid - #__1__

Group III Conditioner-L.D.Caulk Co. --- #__2__

The above numbers have been assigned without the knowledge of the principle operator, and will not be released to the operator until the data-gathering phase of this study is completed.

Each amber bottle has had its label changed from the known agent name to its above assigned number.

Committee Chairman _____ Date_____

TABLE I

Effects of 3 conditioning agents at occlusal surface (A-unconditioned occlusal surface; B-post-conditioned occlusal surface) and depth of lesion (C-post-conditioned depth of lesion) measured by positive or negative cultured bacterial growth.

Patient #	Randomly assigned conditioning agent	Universal tooth #	3-day incubation*			4th day incubation**			Subculture***		
			A	B	C	A	B	C	A	B	C
1****	2	2	+	-	-	+	-	-	N/A	-	-
2	2	15	+	-	+	+	-	+	N/A	-	N/A
3	3	3	+	+	-	+	+	+	N/A	N/A	N/A
4	3	31	+	+	+	+	+	+	N/A	N/A	N/A
5	1	14	+	-	+	+	-	+	N/A	-	N/A
6	1	31	+	+	+	+	+	+	N/A	N/A	N/A
7	1	18	+	+	-	+	+	-	N/A	N/A	-

* Culture read after 3 days of anaerobic (37°F) incubation

** Culture transferred to aerobic (37°F) incubation after 3 days. Results read on 4th day.

*** Negative growth after 4 days subcultured and incubated for 3 days aerobically at 37°F.

**** Eliminated due to saliva leakage

	3 days (anaerobic)	4th day (aerobic)
Initial	-	-
Final	-	-

TABLE II

Effects of 3 conditioning agents at occlusal surface (A-unconditioned occlusal surface; B-post-conditioned occlusal surface) and depth of lesion (C-post-conditioned depth of lesion) measured by positive or negative cultured bacterial growth.

Patient #	Randomly assigned conditioning agent	Universal tooth #	3-day incubation*			4th day incubation**			Subculture***		
			A	B	C	A	B	C	A	B	C
8	3	3	+	+	+	+	+	+	N/A	N/A	N/A
9	2	3	+	+	-	+	+	-	N/A	N/A	-
10	2	3	+	+	+	+	+	+	N/A	N/A	N/A
Cancelled											
11	3	3	+	+	+	+	+	+	N/A	N/A	N/A
12	2	3	+	+	+	+	+	+	N/A	N/A	N/A
Cancelled											

* Culture read after 3 days of anaerobic (37°F) incubation

** Culture transferred to aerobic (37°F) incubation after 3 days. Results read on 4th day.

*** Negative growths after 4 days subcultured and incubated for 3 days aerobically at 37°F.

	3 days (anaerobic)	4th day (aerobic)
Initial	-	-
Final	-	-

TABLE III

Effects of 3 conditioning agents at occlusal surface (A-unconditioned occlusal surface; B-post-conditioned occlusal surface) and depth of lesion (C-post-conditioned depth of lesion) measured by positive or negative cultured bacterial growth.

Patient #	Randomly assigned condition-ing agent	Universal tooth #	3-day incubation*			4th day incubation**			Subculture***		
			A	B	C	A	B	C	A	B	C
Failed											
13	2	3	+	+	+	+	+	+	N/A	N/A	N/A
14	3	3	+	+	+	+	+	+	N/A	N/A	N/A
15	3	15	+	+	+	+	+	+	N/A	N/A	N/A
16	3	14	+	+	+	+	+	+	N/A	N/A	N/A
17	1	14	+	+	-	+	+	-	N/A	N/A	-
18	1	30	+	+	+	+	+	+	N/A	N/A	N/A
19	1	19	+	+	+	+	+	+	N/A	N/A	N/A
20	1	19	+	+	+	+	+	+	N/A	N/A	N/A
21	3	19	+	+	+	+	+	+	N/A	N/A	N/A
22	2	3	+	+	+	+	+	+	N/A	N/A	N/A
23	3	14	+	+	+	+	+	+	N/A	N/A	N/A
24	3	3	+	+	+	+	+	+	N/A	N/A	N/A
25	2	14	+	+	+	+	+	+	N/A	N/A	N/A

* Culture read after 3 days of anaerobic (37°F) incubation.

** Culture transferred to aerobic (37°F) incubation after 3 days. Results read on 4th day.

*** Negative growths after 4 days subcultured and incubated for 3 days aerobically at 37°F.

	3 days (anaerobic)	4th day (aerobic)
Initial	-	-
Final	+	+

TABLE IV

Effects of 3 conditioning agents at occlusal surface (A-unconditioned occlusal surface; B-post-conditioned occlusal surface) and depth of lesion (C-post-conditioned depth of lesion) measured by positive or negative cultured bacterial growth.

Patient #	Randomly assigned conditioning agent	Universal tooth #	3-day incubation*			4th day incubation**			Subculture***		
			A	B	C	A	B	C	A	B	C
26	3	3	+	+	+	+	+	+	N/A	N/A	N/A
Dropped		15									
Dropped		15									
27	3	30	+	+	+	+	+	+	N/A	N/A	N/A
28	2	3	+	-	-	+	-	-	N/A	-	-
29	2	30	+	-	+	+	-	+	N/A	-	N/A
30	1	30	+	+	+	+	+	+	N/A	N/A	N/A

* Culture read after 3 days of anaerobic (37°F) incubation.

** Culture transferred to aerobic (37°F) incubation after 3 days. Results read on 4th day.

*** Negative growths after 4 days subcultured and incubated for 3 days aerobically at 37°F.

	3 days (anaerobic)	4th day (aerobic)
Initial	-	-
Final	-	-

TABLE V

Effects of 3 conditioning agents at occlusal surface (A-unconditioned occlusal surface; B-post-conditioned occlusal surface) and depth of lesion (C-post-conditioned depth of lesion) measured by positive or negative cultured bacterial growth.

Patient #	Randomly assigned conditioning agent	Universal tooth #	3-day incubation*			4th day incubation**			Subculture***		
			A	B	C	A	B	C	A	B	C
31	3	14	+	+	+	+	+	+	N/A	N/A	N/A
32	2	19	+	+	+	+	+	+	N/A	N/A	N/A
33	2	3	+	+	+	+	+	+	N/A	N/A	N/A
34	1	14	+	-	-	+	+	+	N/A	N/A	N/A
Failed Cancelled											
35	2	31	+	+	+	+	+	+	N/A	N/A	N/A

* Culture read after 3 days of anaerobic (37°F) incubation

** Culture transferred to aerobic (37°F) incubation after 3 days. Results read on 4th day.

*** Negative growths after 4 days subcultured and incubated for 3 days aerobically at 37°F.

	3 days (anaerobic)	4th day (aerobic)
Initial	-	-
Final	-	-

TABLE VI

Effects of 3 conditioning agents at occlusal surface (A-unconditioned occlusal surface; B-post-conditioned occlusal surface) and depth of lesion (C-post-conditioned depth of lesion) measured by positive or negative cultured bacterial growth.

Patient #	Randomly assigned conditioning agent	Universal tooth #	3-day incubation*			4th day incubation**			Subculture***		
			A	B	C	A	B	C	A	B	C
36	3	3	+	+	+	+	+	+	N/A	N/A	N/A
37	1	14	+	-	-	+	-	-	N/A	-	-
Eliminated											
38	2	3	+	+	+	+	+	+	N/A	N/A	N/A
39	3	2	+	+	+	+	+	+	N/A	N/A	N/A
Failed											
40	2	18	+	+	+	+	+	+	N/A	N/A	N/A
41	2	14	+	+	+	+	+	+	N/A	N/A	N/A

* Culture read after 3 days of anaerobic (37°F) incubation

** Culture transferred to aerobic (37°F) incubation after 3 days. Results read on 4th day.

*** Negative growths after 4 days subcultured and incubated for 3 days aerobically at 37°F.

	3 days (anaerobic)	4th day (aerobic)
Initial	-	-
Final	-	-

TABLE VII

Effects of 3 conditioning agents at occlusal surface (A-unconditioned occlusal surface; B-post-conditioned occlusal surface) and depth of lesion (C-post-conditioned depth of lesion) measured by positive or negative cultured bacterial growth.

Patient #	Randomly assigned conditioning agent	Universal tooth #	3-day incubation*			4th day incubation**			Subculture***		
			A	B	C	A	B	C	A	B	C
Cancelled											
Cancelled											
41	2	14	+	+	+	+	+	+	N/A	N/A	N/A
42	2	3	+	+	-	+	+	-	N/A	N/A	-
43	3	3	+	+	+	+	+	+	N/A	N/A	N/A
44	1	3	+	+	+	+	+	+	N/A	N/A	N/A
45	1	30	+	+	+	+	+	+	N/A	N/A	N/A
Failed											
46	2	3	+	-	+	+	-	+	N/A	-	N/A
Failed											
47	2	19	+	-	+	+	-	+	N/A	-	N/A
48	1	3	+	+	-	+	+	-	N/A	N/A	-
49	3	18	+	+	+	+	+	+	N/A	N/A	N/A
50	2	30	+	+	+	+	+	+	N/A	N/A	N/A

* Culture read after 3 days of anaerobic (37°F) incubation

** Culture transferred to aerobic (37°F) incubation after 3 days. Results read on 4th day.

*** Negative growths after 4 days subcultured and incubated for 3 days aerobically at 37°F.

	3 days (anaerobic)	4th day (aerobic)
Initial	-	-
Final	-	-

TABLE VIII

Effects of 3 conditioning agents at occlusal surface (A-unconditioned occlusal surface; B-post-conditioned occlusal surface) and depth of lesion (C-post-conditioned depth of lesion) measured by positive or negative cultured bacterial growth.

Patient #	Randomly assigned conditioning agent	Universal tooth #	3-day incubation*			4th day incubation**			Subculture***		
			A	B	C	A	B	C	A	B	C
51	3	3	+	+	+	+	+	+	N/A	N/A	N/A
52	2	30	+	+	+	+	+	+	N/A	N/A	N/A
53	3	15	+	+	+	+	+	+	N/A	N/A	N/A
54	3	15	+	+	+	+	+	+	N/A	N/A	N/A
55	3	30	+	+	+	+	+	+	N/A	N/A	N/A
56	2	14	+	+	-	+	+	-	N/A	N/A	-
57	2	14	+	+	+	+	+	+	N/A	N/A	N/A

* Culture read after 3 days of anaerobic (37°F) incubation

** Culture transferred to aerobic (37°F) incubation after 3 days. Results read on 4th day.

*** Negative growths after 4 days subcultured and incubated for 3 days aerobically at 37°F.

	3 days (anaerobic)	4th day (aerobic)
Initial	-	-
Final	-	-

TABLE IX

Effects of 3 conditioning agents at occlusal surface (A-unconditioned occlusal surface; B-post-conditioned occlusal surface) and depth of lesion (C-post-conditioned depth of lesion) measured by positive or negative cultured bacterial growth.

Patient #	Randomly assigned conditioning agent	Universal tooth #	3-day incubation*			4th day incubation**			Subculture***		
			A	B	C	A	B	C	A	B	C
58	3	2	+	+	+	+	+	+	N/A	N/A	N/A
59	3	19	+	+	+	+	+	+	N/A	N/A	N/A
60	1	19	+	+	+	+	+	+	N/A	N/A	N/A
61	3	19	+	+	+	+	+	+	N/A	N/A	N/A
Cancelled											
62	1	3	+	+	+	+	+	+	N/A	N/A	N/A
63	3	30	+	+	+	+	+	+	N/A	N/A	N/A

* Culture read after 3 days of anaerobic (37°F) incubation

** Culture transferred to aerobic (37°F) incubation after 3 days. Results read on 4th day.

*** Negative growths after 4 days subcultured and incubated for 3 days aerobically at 37°F.

	3 days (anaerobic)	4th day (aerobic)
Initial	-	-
Final	-	-

TABLE X

Effects of 3 conditioning agents at occlusal surface (A-unconditioned occlusal surface; B-post-conditioned occlusal surface) and depth of lesion (C-post-conditioned depth of lesion) measured by positive or negative cultured bacterial growth.

Patient #	Randomly assigned condition-ing agent	Universal tooth #	3-day incubation*			4th day incubation**			Subculture***		
			A	B	C	A	B	C	A	B	C
64	1	19	+	+	+	+	+	+	N/A	N/A	N/A
65	1	19	+	+	+	+	+	+	N/A	N/A	N/A
Cancelled											
66	3	19	+	+	+	+	+	+	N/A	N/A	N/A
Cancelled											
67	3	3	+	+	+	+	+	+	N/A	N/A	N/A
68	3	3	+	+	+	+	+	+	N/A	N/A	N/A

* Culture read after 3 days of anaerobic (37°F) incubation

** Culture transferred to aerobic (37°F) incubation after 3 days. Results read on 4th day.

*** Negative growths after 4 days subcultured and incubated for 3 days aerobically at 37°F.

	3 days (anaerobic)	4th day (aerobic)
Initial	-	-
Final	-	-

TABLE XI

Effects of 3 conditioning agents at occlusal surface (A-unconditioned occlusal surface; B-post-conditioned occlusal surface) and depth of lesion (C-post-conditioned depth of lesion) measured by positive or negative cultured bacterial growth.

Patient #	Randomly assigned conditioning agent	Universal tooth #	3-day incubation*			4th day incubation**			Subculture***		
			A	B	C	A	B	C	A	B	C
Failed											
69	1	3	+	+	+	+	+	+	N/A	N/A	N/A
70	3	19	+	+	+	+	+	+	N/A	N/A	N/A
71	3	14	+	+	+	+	+	+	N/A	N/A	N/A
72	1	31	+	+	+	+	+	+	N/A	N/A	N/A
73	1	30	+	+	+	+	+	+	N/A	N/A	N/A
74	3	19	+	+	+	+	+	+	N/A	N/A	N/A
75	1	14	+	-	-	+	-	-	N/A	-	-
76	3	14	+	+	+	+	+	+	N/A	N/A	N/A
Cancelled											
77	2	30	+	+	+	+	+	+	N/A	N/A	N/A
78	2	14	+	+	+	+	+	+	N/A	N/A	N/A
79	3	15	+	+	+	+	+	+	N/A	N/A	N/A
80	3	14	+	+	+	+	+	+	N/A	N/A	N/A

* Culture read after 3 days of anaerobic (37°F) incubation

** Culture transferred to aerobic (37°F) incubation after 3 days. Results read on 4th day.

*** Negative growths after 4 days subcultured and incubated for 3 days aerobically at 37°F.

	3 days (anaerobic)	4th day (aerobic)
Initial	-	-
Final	-	-

TABLE XII

Effects of 3 conditioning agents at occlusal surface (A-unconditioned occlusal surface; B-post-conditioned occlusal surface) and depth of lesion (C-post-conditioned depth of lesion) measured by positive or negative cultured bacterial growth.

Patient #	Randomly assigned conditioning agent	Universal tooth #	3-day incubation* A	B	C	4th day incubation** A	B	C	Subculture*** A	B	C
Cancelled											
81	1	30	+	+	+	+	+	+	N/A	N/A	N/A
82	2	19	+	+	-	+	+	-	N/A	N/A	-
83	2	18	+	+	+	+	+	+	N/A	N/A	N/A
84	1	14	+	-	+	+	-	+	N/A	-	N/A
Cancelled											
85	3	19	+	+	+	+	+	+	N/A	N/A	N/A

* Culture read after 3 days of anaerobic (37°F) incubation

** Culture transferred to aerobic (37°F) incubation after 3 days. Results read on 4th day.

*** Negative growths after 4 days subcultured and incubated for 3 days aerobically at 37°F.

	3 days (anaerobic)	4th day (aerobic)
Initial	-	-
Final	-	-

TABLE XIII

Effects of 3 conditioning agents at occlusal surface (A-unconditioned occlusal surface; B-post-conditioned occlusal surface) and depth of lesion (C-post-conditioned depth of lesion) measured by positive or negative cultured bacterial growth.

Patient #	Randomly assigned condition-ing agent	Universal tooth #	3-day incubation*			4th day incubation**			Subculture***		
			A	B	C	A	B	C	A	B	C
86	1	3	+	-	+	+	-	+	N/A	-	N/A
87	2	19	+	+	+	+	+	+	N/A	N/A	N/A
Cancelled											
88	2	19	+	+	+	+	+	+	N/A	N/A	N/A
89	2	19	+	-	+	+	-	+	N/A	-	N/A
90	1	19	+	-	+	+	-	+	N/A	-	N/A
91	3	19	+	+	+	+	+	+	N/A	N/A	N/A

* Culture read after 3 days of anaerobic (37°F) incubation

** Culture transferred to aerobic (37°F) incubation after 3 days. Results read on 4th day.

*** Negative growths after 4 days subcultured and incubated for 3 days aerobically at 37°F.

	3 days (anaerobic)	4th day (aerobic)
Initial	-	-
Final	-	-

TABLE XIV

Effects of 3 conditioning agents at occlusal surface (A-unconditioned occlusal surface; B-post-conditioned occlusal surface) and depth of lesion (C-post-conditioned depth of lesion) measured by positive or negative cultured bacterial growth.

Patient #	Randomly assigned conditioning agent	Universal tooth #	3-day incubation*			4th day incubation**			Subculture***		
			A	B	C	A	B	C	A	B	C
92 Failed	2	14	+	+	-	+	+	-	N/A	N/A	-
93	2	31	+	+	+	+	+	+	N/A	N/A	N/A
94 Cancelled	1	30	+	+	+	+	+	+	N/A	N/A	N/A
95 Cancelled	3	30	+	+	+	+	+	+	N/A	N/A	N/A

* Culture read after 3 days of anaerobic (37°F) incubation

** Culture transferred to aerobic (37°F) incubation after 3 days. Results read on 4th day.

*** Negative growths after 4 days subcultured and incubated for 3 days aerobically at 37°F.

	3 days (anaerobic)	4th day (aerobic)
Initial	-	-
Final	-	-

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EFFECT OF SEALANT CONDITIONERS ON OCCLUSAL SURFACE BACTERIA: A CLINICAL STUDY

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This clinical study evaluated the effects of conditioning agents for pit and fissure sealants on the bacteria present in occlusal grooves and fissures in permanent molars. The conditioning agents, 50 percent phosphoric acid and 50 percent phosphoric acid attenuated with 7 percent zinc oxide, with distilled sterile water being used as a control, were compared for their ability to kill bacteria in carious occlusal lesions. Eighty-six teeth from children eight to twelve years of age were conditioned with one of the randomly assigned agents using a blind method to prevent bias. The teeth were then cultured with a method that measured results as to growth or no growth after incubation. The culturing was done at both the occlusal surface and the depth of the lesion.

A chi square test demonstrated that there were no significant differences between the conditioners on either the occlusal or depth of the lesion cultures (x^2=.31, df=2, a=.05).

The two conditioners did not totally kill the bacteria in occlusal lesions often enough to justify their use as bactericidal agents before sealants are applied.